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Methylmercury-induced ferroptosis may be attenuated by vitamin K in PC12 cells

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INTRODUCTION

As a ubiquitous environmental pollutant, methylmercury (MeHg) induces toxic effects in the nervous system.

However, the exact mechanism of its neurotoxicity has not been fully elucidated. Ferroptosis may be related to methylmercury toxicity and methylmercury-induced ferroptosis may be attenuated by vitamin K.

METHOD

PC12 cells with neuron-like characteristics were selected and treated with different concentrations of MeHg (0, 1, 2.5, 5, 10 μM) for 6 h. CCK8 was used to detect cell viability, FerroOrange fluorescent probe was used to detect the level of free ferrous ions in cells, microplate method was used to detect the level of reduced GSH in cells, FSP1, SLC7A11 and GPX4 protein expression were detected by western blotting, and the changes of Lipid ROS content in cells were detected by flow cytometry.

In the vitamin K intervention experiment, the MeHg group was treated with 5 μM MeHg for 6 h, the vitamin K + MeHg group was pretreated with vitamin K (0, 10, 20, 40, 80, 100 μM) for 1 h, and then co-treated with 5 μM MeHg for 6 h, the changes of intracellular Lipid ROS content were detected by flow cytometry.

RESULTS

Methylmercury exposure induced PC12 cytotoxicity

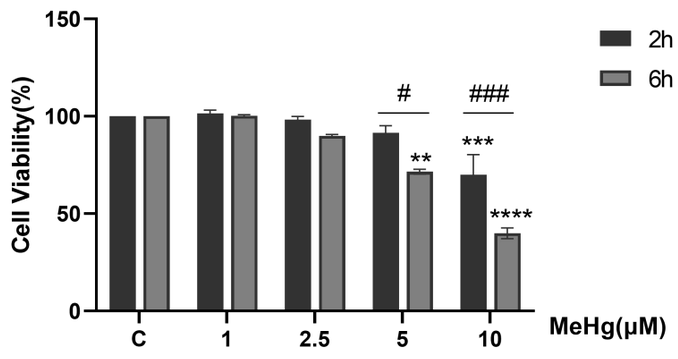
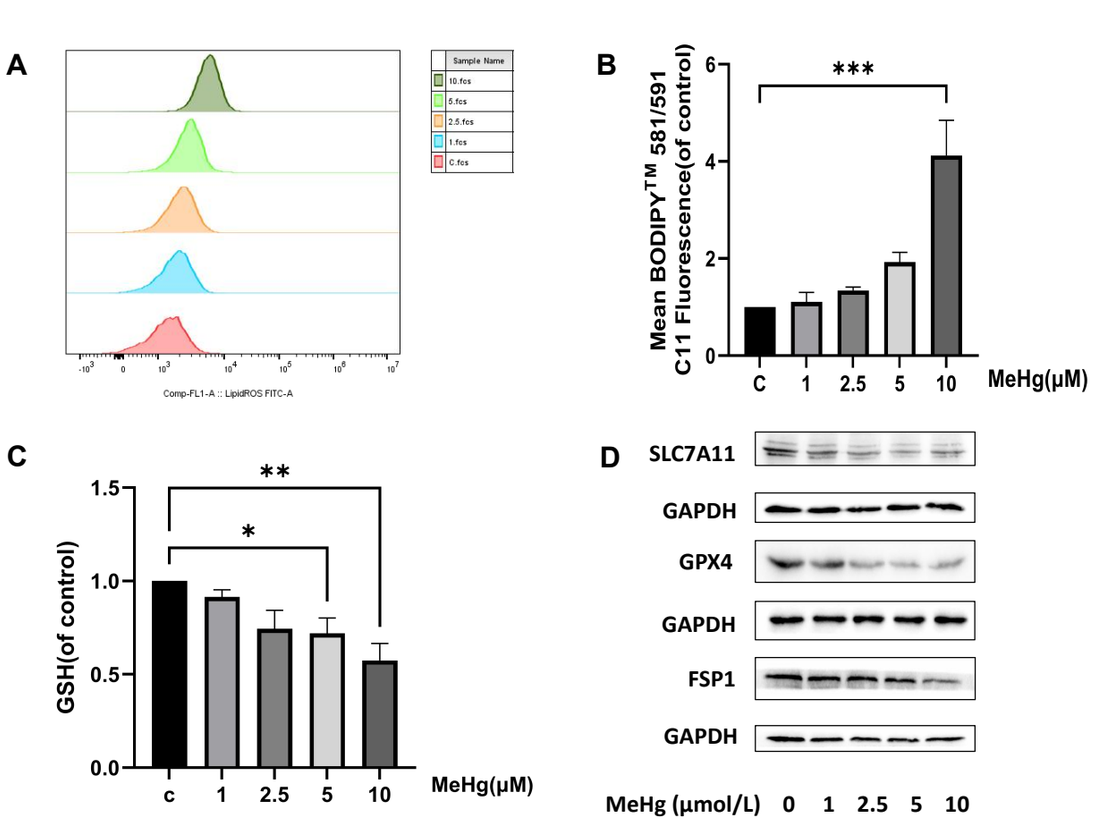


Figure 1. The changes in PC12 cell viability after exposure to different concentrations of methylmercury chloride (0,1,2.5,5,10 μmol/L) for 2 and 6 h.

Methylmercury exposure triggered ferroptosis in PC12 cells



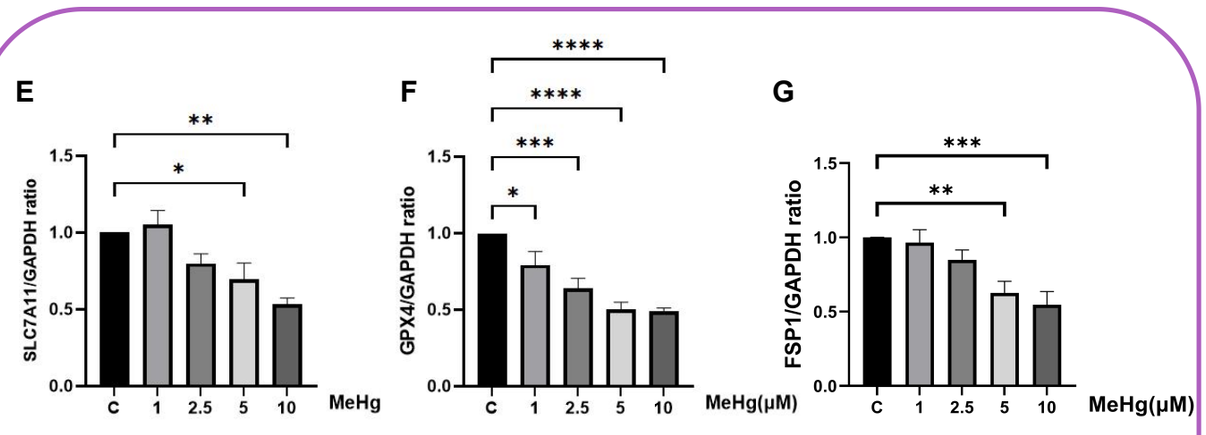


Figure 2. MeHg exposure triggered ferroptosis in PC12 cells: (A-B) The mean fluorescence intensity representing lipid peroxidation in PC12 cells exposed to different concentrations of MeHg (0, 1,2.5,5,10μmol/L) for 6 h was measured using flow cytometry. (C) GSH levels in PC12 cells treated for 6 h with different concentrations of MeHg. (D-G)Effects of different doses of MeHg incubation (0,1,2.5,5,10μmol/L) for 6 h on the ferroptosis-related proteins (SLC7A11,GPX4,FSP1) in the PC12 cells using Western blot.

Vitamin K partially alleviated methylmercury-induced ferroptosis

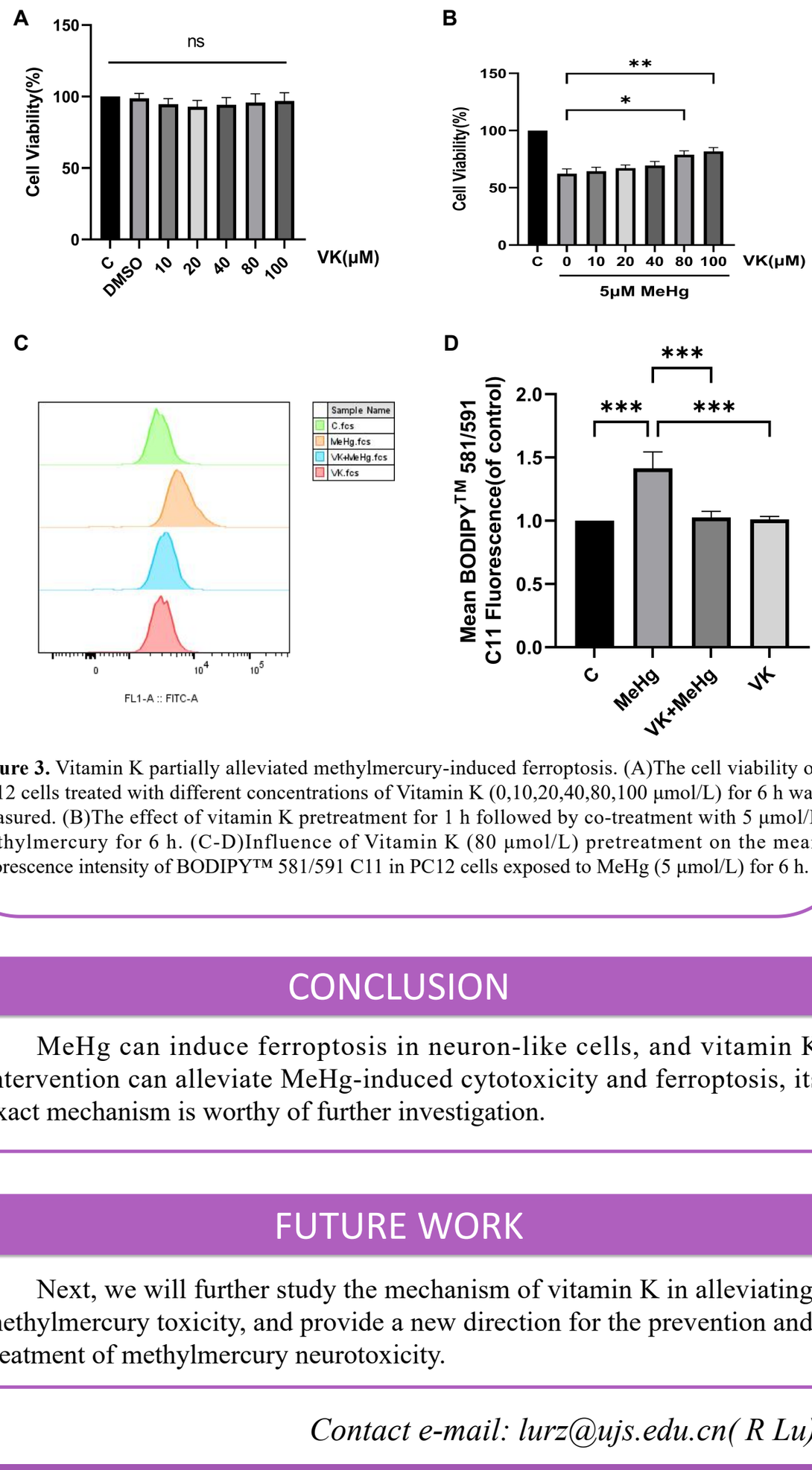


Figure 3. Vitamin K partially alleviated methylmercury-induced ferroptosis. (A)The cell viability of PC12 cells treated with different concentrations of Vitamin K (0,10,20,40,80,100 μmol/L) for 6 h was measured. (B)The effect of vitamin K pretreatment for 1 h followed by co-treatment with 5 μmol/L methylmercury for 6 h. (C-D)Influence of Vitamin K (80 μmol/L) pretreatment on the mean fluorescence intensity of BODIPY™ 581/591 C11 in PC12 cells exposed to MeHg (5 μmol/L) for 6 h.

CONCLUSION

MeHg can induce ferroptosis in neuron-like cells, and vitamin K intervention can alleviate MeHg-induced cytotoxicity and ferroptosis, its exact mechanism is worthy of further investigation.

FUTURE WORK

Next, we will further study the mechanism of vitamin K in alleviating methylmercury toxicity, and provide a new direction for the prevention and treatment of methylmercury neurotoxicity.

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